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**A Study of the Environmental Conditions Influencing
the Development of Stem Rust in the Absence
of an Alternate Host**

III. FURTHER STUDIES OF THE VIABILITY OF THE UREDINIOSPORES
OF PUCCINIA GRAMINIS TRITICI

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INTRODUCTION

In a previous report¹ the author showed that the influence of relative humidity on the viability of the urediniospores of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. Physiologic Form III was very pronounced and closely associated with temperature. At the end of 16 weeks the spores were still viable at the medium humidities and lower temperatures employed, as determined by the percentage of spore germination and the ability of the urediniospores to produce infection on wheat seedlings. With all humidities at higher temperatures the spores were viable only a short time. There appeared to be a direct relation between the viability of the urediniospores and the temperature and relative humidity.

This study has been continued with two other Physiologic Forms of *Puccinia graminis tritici*. During the season of 1922-23 the urediniospores of Physiologic Form IX were used, while the following year an attempt was made to determine how long the urediniospores of Physiologic Form XXI remained viable at the medium relative humidities and the lower temperatures.

METHODS

The same apparatus as previously described was used. Likewise, the same methods were used¹ namely: 7-day old seedlings of Little Club (C. I. No. 4066) were hand inoculated with the urediniospores of the Physiologic Form to be studied and incubated for 48 hours at the optimum temperature for infection. After incubation they were trimmed and

¹Peltier, G. L. A Study of the Environmental Conditions Influencing the Development of Stem Rust in the Absence of an Alternate Host. I. The Viability of the Urediniospores of *Puccinia graminis tritici* Form III. Neb. Agr. Exp. Sta. Res. Bul. 22, 15 pp. 3 figs., 1922.

placed on a bench in a greenhouse held at a temperature of approximately 25° C. Fourteen days after inoculation, the leaves with pustules were cut off and placed on the stands in the dishes previously described.¹ At stated intervals the leaves were withdrawn from the dishes and spore germination tests made. The method used for the germination of the urediniospores was modified somewhat in order to obtain more uniform results. In the preliminary experiments the spores were placed in a drop of water on a cover glass and inverted over a glass ring, mounted on a slide. It was found that much better and more uniform results were obtained by dusting the spores onto a drop of water in a Syracuse watch glass, so that the spores floated on top of the drop. All the germination tests were run in triplicate. The same counting methods were used to obtain the percentage of spore germination.

In the greenhouse 6 or 12 seedling wheat plants (Little Club C. I. No. 4066) were inoculated with the spore material remaining after the spore germination tests were made. Fourteen days after inoculation the number of leaves infected together with the number of pustules on each leaf were recorded.

EXPERIMENTAL DATA

VIABILITY OF THE UREDINIOSPORES OF PHYSIOLOGIC FORM IX

Germination tests.—The same set of 6 temperatures (5°, 10°, 15°, 20°, 25° and 30° C.) and 11 relative humidities (from 0 to 100 per cent at approximately 10 per cent intervals) were employed, as in the previous report.¹ Owing to the fact that none of the spores of Physiologic Form IX were viable at the end of the first week in the first trial at any relative humidity when held at 30° C. and only a low percentage of germination over a short period of time was obtained at some of the medium relative humidities when maintained at 25°, daily germination tests of spores held at these two temperatures were made. The results are listed in Table 1. The results show very clearly that at 30° C., with relative humidities between 60.7 and 100 per cent, all the spores were dead at the end of 9 days. Likewise, the spores were not viable after 10 days at the lowest 2 relative humidities. However, at the medium relative humidities the spores were viable for about 1 week longer or a total of about 2½ weeks.

¹ L. C.

TABLE 1.—*Viability of the urediniospores of Puccinia graminis tritici Physiologic Form IX*

Approximate relative humidity	After 1 day	After 2 days	After 3 days	After 4 days	After 6 days	After 7 days	After 8 days	After 9 days	After 10 days	After 11 days	After 13 days	After 14 days	After 15 days	After 16 days	After 17 days	After 19 days	After 20 days	After 21 days	After 22 days	After 24 days	After 28 days	After 35 days	After 42 days
<i>30° C.</i>																							
Per cent																							
100.0	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
89.9	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
80.5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
70.4	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
60.7	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
49.0	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
38.0	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
29.5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
21.5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
10.5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
0.0	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
<i>25° C.</i>																							
Per cent																							
100.0	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
89.9	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
80.5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
70.4	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
60.7	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
49.0	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
38.0	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
29.5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
21.5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
10.5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
0.0	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++

0 = No germination.
 — = 1 to 5 per cent germination.
 ± = 5 to 10 per cent germination.
 ++ = 10 to 40 per cent germination.
 +++ = above 50 per cent germination.

TABLE 2.—*Viability of the urediniospores of Puccinia graminis tritici Physiologic Form IX*

Approximate relative humidity	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks	After 6 weeks	After 7 weeks	After 8 weeks	After 9 weeks	After 10 weeks	After 11 weeks	After 12 weeks	After 13 weeks	After 14 weeks	After 15 weeks	After 16 weeks	After 17 weeks	After 18 weeks
Per cent	20° C.																	
100.0	##	—	—	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89.9	##	—	—	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
70.4	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
60.7	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
49.0	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
38.0	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.0	+	—	—	—	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Per cent	15° C.																	
100.0	##	—	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89.9	##	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
80.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
70.4	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
60.7	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
49.0	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
38.0	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.0	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Per cent	10° C.																	
100.0	##	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
89.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
80.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
70.4	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
60.7	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
49.0	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
38.0	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.0	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Per cent	5° C.																	
100.0	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
89.9	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
80.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
70.4	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
60.7	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
49.0	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
38.0	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10.5	++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.0	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Material exhausted.

0 = No germination.

— = 1 to 5 per cent germination.

+ = 5 to 10 per cent germination.

++ = 10 to 40 per cent germination.

+++ = above 50 per cent germination.

As stated above, no urediniospores were viable at the end of the first week at any relative humidity at 30° C. when weekly tests were made. However, when daily germination tests were run, the spores retained their viability at the medium relative humidities for as long as 19 days. In fact only those spores held at 100 per cent relative humidity were not viable at the end of 7 days. Apparently the removal of the lid from the dishes long enough to obtain the material necessary for the daily test had some influence on the viability of the spores. Similar results were noted at 25° C., especially at the medium relative humidities.

The relation between the viability of the spores and the relative humidity at 25° C. was the same as at 30° except that the spores were viable for a longer period of time and over a greater range of relative humidities. At the end of 5 weeks a few spores were still viable at two of these relative humidities.

The results of the weekly germination tests obtained at the temperatures of 5°, 10°, 15°, and 20° C. are listed in Table 2. Comparing the results obtained with Physiologic Form IX with those previously reported for Physiologic Form III it would appear that the spores of Physiologic Form IX were viable over a longer period of time at all temperatures and relative humidities than Physiologic Form III. The method used for germinating the urediniospores of Physiologic Form IX may account in part for this difference. However, the same general relation between the viability of the urediniospores and the relative humidity and temperature was maintained.

Infection Tests.—In order to check the results of the germination tests, 6 plants were inoculated each week from material held at each temperature and at each relative humidity. The results are listed in Table 3 and for the most part are a complete check on the germination tests. While the number of pustules per leaf are not listed in the table, as a rule the higher the percentage of infection obtained the greater the number of pustules per leaf were recorded. As a rule infection was obtained when 10 per cent or more of the spores were viable. In some instances infection was not obtained when a higher per cent of spores were viable. These may be accounted for in part to the lack of proper conditions during incubation, cloudy weather, etc.

TABLE 3.—*Number of plants out of 6 infected with urediniospores of Puccinia graminis tritici Physiologic Form IX after being exposed to various temperatures and relative humidities for different periods of time*

Approximate relative humidity	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks	After 6 weeks	After 7 weeks	After 8 weeks	After 9 weeks	After 10 weeks	After 11 weeks	After 12 weeks	After 13 weeks	After 14 weeks	After 15 weeks	After 16 weeks	After 17 weeks	After 18 weeks
20° C.																		
Per cent																		
100.0
89.9
80.5
70.4	1
60.7	1	...	1	1	1
49.0	1	1	1	1	...	3	1	*	...
38.0	1	2	1	...	4	2	1
29.5	2
21.5	1
10.5
0.0
15° C.																		
Per cent																		
100.0	3
89.9	1
80.5
70.4
60.7	3	1	...	2	...	1	1	*
49.0	2	...	1	2	1	2	...	1	...	2	...	2	1	...	*
38.0	1	...	1	1	2	1	...	3	4	4
29.5	1	1	1	1	2
21.5	1
10.5
0.0	1
10° C.																		
Per cent																		
100.0
89.9
80.5	1
70.4	1	1
60.7	1	1	1	1	3	1	2	1	3	1	...
49.0	3	1	1	...	1	...	1	...	1	4	...	4	4	...	1
38.0	3	...	1	2	1	...	4
29.5	2	1
21.5	1
10.5	1	1
0.0
5° C.																		
Per cent																		
100.0
89.9	...	1	1	1
80.5
70.4	3	...	2	1	2	...	4	5	...	4	...	3	1	2	*
60.7	1	1	3	1	...	1	5	...	4	3	5	2
49.0	1	2	...	2	3	5	...	3	3	4	3
38.0	4	4	1	2	1
29.5	3	1	...	1
21.5	2	...	1	3
10.5
0.0	1

* Material exhausted.

VIABILITY OF THE UREDINIOSPORES OF PHYSIOLOGIC FORM XXI

Germination Tests.—In this experiment enough material was put into the dishes to last one year, when bimonthly tests were made. Only the temperatures of 5°, 10°, 15°, and 20° C. were employed together with the relative humidities between 29.5 and 70.4 per cent. This series of relative humidities has been previously referred to as the medium relative humidities.

The results of this experiment are listed in Table 4. A glance at the table shows that the same general relations exist as have been previously pointed out; namely, with increasing temperature the viability of the spores decreases irrespectively of the relative humidities at which the spores are maintained. At any stated temperature the viability of the spores is greatest at the medium relative humidities, while the optimum for spore viability is at 49.0 per cent relative humidity. The decline in the viability of the spores at the lower relative humidities is not as rapid as at the higher humidities, but nevertheless is faster than that at the medium relative humidities. It is interesting to note that urediniospores have been carried thru, under the conditions of the experiment, for 52 weeks at a temperature of 5° C. and at a relative humidity of 49.0 per cent. At the end of this period germination tests showed that 30 per cent or more of the spores were still viable.

Infection Tests.—Greater care was observed in maintaining the proper conditions for incubation in this experiment. Artificial light was used during cloudy days. The number of plants inoculated was also increased from 6 to 12, and with more inoculum at hand all conditions for successful infection of the plants were as ideal as possible. As a consequence a very high percentage of infection was obtained thruout the experiment. The results of this infection test are given in Table 5.

In many instances it will be noted that the infection tests proved that the spores were viable for a longer period of time than was determined by means of the germination studies. As a rule, however, the same general results were obtained, which need not be discussed here except to point out that spores held at 5° C. and at 49.0 per cent relative humidity for 52 weeks produced a rather heavy infection on 2 out of 12 plants inoculated.

STEM RUST IN THE ABSENCE OF AN ALTERNATE HOST 11

TABLE 5.—Number of plants out of 12 infected with the urediniospores of *Puccinia graminis tritici* Physiologic Form XXI after being exposed to various temperatures and relative humidities for different periods of time

Approximate relative humidity	After 2 weeks	After 4 weeks	After 6 weeks	After 8 weeks	After 10 weeks	After 12 weeks	After 14 weeks	After 16 weeks	After 18 weeks	After 20 weeks	After 22 weeks	After 24 weeks	After 26 weeks	After 28 weeks ¹	After 52 weeks
20° C.															
Per cent															
70.4	5	4	3	1	8	1	3	1
60.7	8	6	1	3	2	12	3	2
49.0	9	5	7	3	8	8	3	1	3	6	1
38.0	9	7	5	6	10	8	3	2
29.5	9	2	8	7	7	9	1	2
15° C.															
Per cent															
70.4	10	4	1	8	4	5	3
60.7	8	2	6	11	11	11	3	2
49.0	10	3	3	9	11	12	12	9	1	2	3	2	1
38.0	8	5	4	9	9	12	4	3	7	4	5	1	2
29.5	8	7	4	3	12	12	11	7	9	6	11	1	7
10° C.															
Per cent															
70.4	12	7	2	7	11	11	4	4	5	2	1	2
60.7	6	3	3	11	12	12	12	12	8	11	10	8	9	1
49.0	7	5	4	10	12	12	11	11	12	11	12	11	9	3
38.0	4	4	12	10	11	11	11	12	7	10	11	9	5
29.5	2	2	5	11	12	12	10	9	11	10	12	6	11	1
5° C.															
Per cent															
70.4	6	1	3	2	10	10	11	10	10	1	6	5	4	3
60.7	2	2	4	8	12	12	12	12	12	8	11	10	9	4
49.0	1	2	8	12	12	12	12	12	12	12	7	11	12	9	2
38.0	0	2	1	1	11	12	10	11	12	3	6	10	10	1
29.5	2	6	5	8	10	8	11	7	1	11	3	8	3

¹Temperatures in greenhouse too high for good incubation and subsequent infection. Experiment discontinued until fall.

DISCUSSION

The writer is unable to offer any explanation of the underlying principle involved in this study nor is he able to correlate the results obtained with what actually happens under field conditions other than what was stated in a preliminary report.¹

The results of this study indicate a convenient method for holding rust material over a long period with a considerable saving in time and insuring the purity of the Physiologic Forms, especially when a large number are being carried on stock plants in the greenhouse. Further it solves the problem of carrying rust thru the summer, when the greenhouse temperatures are exceedingly high.

¹ L. C.

For example during the season of 1924, approximately 140 collections of *Puccinia graminis tritici* were made in Nebraska. As this material was collected it was sent directly to Lincoln, where the collections were immediately placed in the chambers previously described, maintained at approximately 49.0 per cent relative humidity. These dishes were placed in a cold storage room held at near 5° C.

This material was held in this room until the outside temperatures were low enough so that fairly constant greenhouse temperatures could be maintained. Stock from these rust collections was then built up when time permitted, and was extended well on into January. Of the 140 collections, only about 10 per cent were lost thru storage.

When a Physiologic Form was obtained from any of the collected material, it was immediately put away in the chambers in cold storage, so that the purity of these forms could be maintained. The amount of routine labor saved in not having to carry these forms continually in the greenhouse can only be appreciated by those who have had to do this work.

SUMMARY

1. The influence of relative humidity and temperature on the viability of the urediniospores of two Physiologic Forms of *Puccinia graminis tritici* has been determined.

2. The same general relation between the viability of the urediniospores and the relative humidity and temperature was found, namely, that the lower the temperature the longer the spores retained their viability at all relative humidities, while at any stated temperature the spores were viable the longest at the medium humidities.

3. Urediniospores of Physiologic Form XXI held at 49.0 per cent relative humidity and maintained at a temperature of 5° C. when tested for germination at the end of 1 year yielded 30 per cent germination and produced a heavy infection on 2 out of 12 plants.

4. The application of this method for the storing of rust material for long periods is discussed.